Final Report

Heterodimeric Ligand: Potential Multidrug-Resistant *Staphylococcus aureus* Therapeutics

By Assistant Prof. Chutima Jiarpinitnun

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This project granted by the Thailand Research Fund

Abstract

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Project Title: **Heterodimeric Ligand: Potential Multidrug-Resistant *Staphylococcus aureus* Therapeutics**

Investigator: **Assistant Prof. Chutima Jiarpinitnun**

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Project Period: **2 years**

Abstract:

The emergence of antibiotic resistance in pathogenic bacteria *Staphylococcus aureus* (*S. aureus*) and its alarming spread has remarkably become global healthcare problem. The antibiotic resistance has complicated the treatment of infections, especially, methicillin-resistant *S. aureus* (MRSA) that acquired resistance to commonly used beta-lactam antibiotics such as penicillin. One method to overcome the resistance is to explore the possibility of using anti-bacterial agents in combination. The successful combination therapy to treat *S. aureus* and MRSA infections is sulfamethoxazole and trimethoprim. However, rises of sulfa-resistance, trimethoprim resistance, and resistance to the combination therapy, in some cases, have called for new antibacterial agents. In this proposed investigation, we will exploit the concept of dimerization to overcome antibiotics resistance. Sulfonamide was designed to covalently link to trimethoprim. This synthetic dimeric ligand was tested for its ability to inhibit *S. aureus* and multidrug- resistant *S. aureus*. We envisioned that the designed dimer could mimick the intermediate adduct of dihydropteroate synthase (DHPS) enzyme, which is molecular target of sulfonamide. In addition, the benzene sulfonamide portion of the dimer could participate in the non-covalent interactions to unique hydrophobic pocket of *S. aureus* dihydrofolate reductase (DHFR), a molecular target of trimethoprim. Hence, we anticipated the ability of the dimeric ligand to overcome the drug resistance.

Keywords: **Multidrug-resistant *Staphylococcus aureus*, Dimeric ligand, Sulfa-drugs, Trimethoprim**

Executive Summary:

Since its discovery in 1880, *Staphylococcus aureus* (*S. aureus*) has continued posting threats to human health. The pathogen could infect not only in human but also in farm animals. In human, *S. aureus* commonly cause uncomplicated skin and soft tissue infections. However, the infection could lead to diseases such as encephalitis, meningitis, and sepsis, which could be fetal. *S. aureus* is often found as a leading cause of subclinical and chronic mastitis in farm animals such as bovine, affecting dairy product quality and potentially agricultural industries. *S. aureus* infections have resulted in tremendous impacts to our economy with regards to healthcare expenses and also indirect impact to food and agricultural industries

The emergence of antibiotic resistance in pathogenic bacteria *S. aureus* and its alarming spread has remarkably become global healthcare problem. The antibiotic resistance has complicated the treatment of infections, especially, methicillin-resistant *S. aureus* (MRSA) that acquired resistance to commonly used beta-lactam antibiotics such as penicillin. MRSA is responsible for up to 60% of cases based on the US National Nosocomial Infections Surveillance System report in 2004 (1). In Thailand, based on a small survey conducted during 1998-2001 reported approximately 35% of hospital-acquired infections were due to MRSA (2). In terms of MRSA treatments, resistance to common antibiotic therapy (e.g. use of penicillins, cephalosporins, erythromycin and combinations thereof) as well as to vancomycin, a “last line of defense” antibiotic have been increasingly reported. There is a clear need to develop new molecules to treat MRSA—therapies that have a reduced likelihood for development of resistance. One method to overcome the resistance is to explore the possibility of using anti-bacterial agents in combination. The successful combination therapy to treat *S. aureus* and MRSA infections is sulfamethoxazole and trimethoprim. However, rises of sulfa-resistance, trimethoprim resistance, and resistance to the combination therapy, in some cases, have called for new antibacterial agents.

The proposed investigation covered the design and syntheses of the dimeric ligands displaying sulfonamide and trimethoprim. These compounds were tested for their abilities to inhibit growth of MSSA (*S. aureus* ATCC control strains 25923 and 29213) and global predominant multidrug-resistant *S. aureus* (USA300 strain SF8300). The susceptibility tests used in this study were disk diffusion assay and microdilution assay. The experiments were conducted side by side with the monomeric counterparts in order to compare their potencies and to determine the effect of dimerization. Our results promisingly indicated that the complex TMP-dimerized sulfonamide could successfully inhibit growth of S. aureus, the resistant strain-MRSA PFGE strain type USA300 (SF8300), a predominant community-associated MRSA (CA-MRSA) strain that causes epidemic outbreaks in several continents, and also the sulfa-resistant MRSA COL strain with the excellent minimum inhibitory concentration (MIC) of approximately 40-fold enhancement when compared to its corresponding monomeric sulfonamide.

Objective:

In this investigation, we exploited the concept of dimerization to overcome antibiotics resistance. Sulfonamide was designed to covalently link to trimethoprim. We envisioned that the dimeric displayed antibacterial agents could enhance efficacy of the monomeric agents. The scope of our investigation would focus on global problematic pathogen *S. aureus*, particularly methicillin-susceptible *S. aureus* (MSSA) and multidrugs-resistant *S. aureus*.

To achieve the objective, we followed these specific aims:

Aim 1. To design and synthesis dimeric ligand displaying sulfonamide and trimethoprim. The length of the linker between sulfonamide and trimethoprim will be varied to determine the effect of the linker to the efficacy observed.

Aim 2. To test for the ability of synthetic dimer to inhibit growth of MSSA and global predominant multidrug-resistant S. aureus. The susceptibility tests used in this study are disk diffusion assay and microdilution assay.

Aim 3. To investigate the mechanism of action underlying the growth inhibition activity.

Research Methodology:

Known antibacterial agents with sulfonamide core such as sulfamethoxazole, sulfapyridine, and sulfadimethoxine typically functions in bacterial growth inhibition by interfering with bacterial folate pathway. Sulfonamide agents function as substrate inhibitor of dihydropteroate synthase (DHPS) by structurally mimicking the key interactions between p-aminobenzoic acid (PABA), essential endogenous substrate of DHPS. DHPS enzyme functions in catalysis of dihydropteroate diphosphate, yielding dihydropteroic acid, a key intermediate for thymidine synthesis. Disruption of DHPS would result in the inability to synthesize thymidine monophosphate (dTMP) for DNA and RNA syntheses; thereby, bacteria would not be able to multiply themselves during cell division. The simplicity in structure of this class of antibiotics ease the production in large scale, thereby low cost. However, the infrequent use of this antibiotics is reportedly due to the quickly developed resistance.

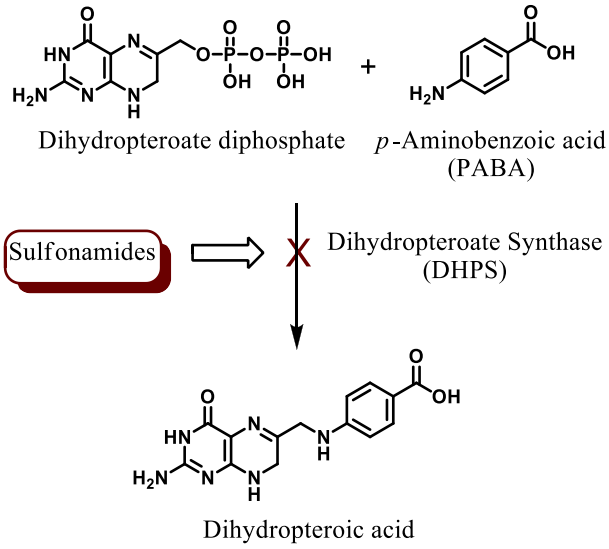


Figure 1. Mode of action of classical sulfonamide antibacterial agents

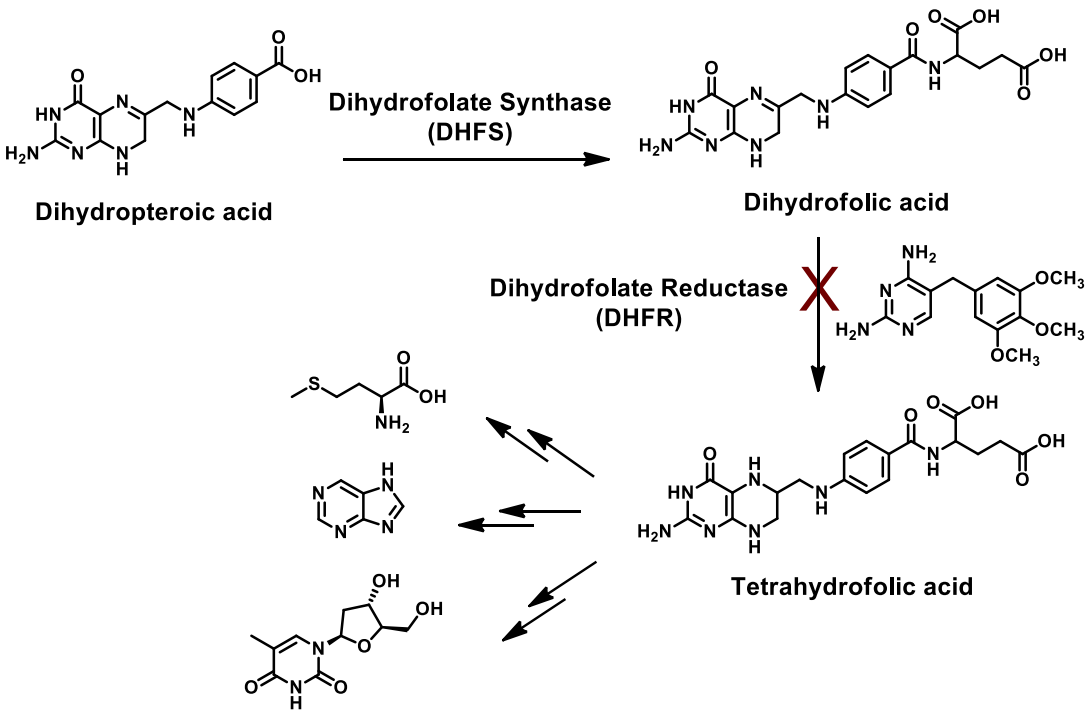


Figure 2. Mode of action of trimethoprim

To overcome the resistance of sulfonamide antibiotics, often sulfa drug is used in combination with trimethoprim for the treatment of bacterial infections caused by *S. aureus*. Sulfamethoxazole and trimethoprim were reported function synergistically, resulting in dramatic enhancement in the ability to inhibit bacterial growth when use in combination. While sulfonamide drug inhibits DHPS enzyme during the early step of folate biosynthetic pathway, trimethoprim interferes with the function of dihydrofolate reductase (DHFR) enzyme, required for the subsequent step of folate synthesis. However, there are numbers of isolates that currently are resistant to both antibacterial agents as well as the combination therapy.

We are interested in the use of dimerization concept to enhance efficacy and overcome bacterial resistance. The concept of dimerization has been well studied in the area of cell surface signaling. Several reports previously highlighted the effects of dimerization in the enhancement of anti-bacterial activity. For example, dimeric vancomycin could overcome vancomycin resistance as it exhibited ability to inhibit bacterial growth of vancomycin-resistant Entercocci (VRE) and vancomycin-resistant *S. aureus* (VRSA) with higher potency than that of single molecular of vancomycin (3). Besides the homodimeric ligand, heterodimeric displayed two different antimicrobial agents were also studied. For example, the glycopeptide-β-lactams heterodimer was synthesized and investigated in antimicrobial activity. Cephalosporin was covalently linked to vancomycin moiety and this covalently displayed two agents in one molecule could increase antibacterial activity and overcome the resistance (4).

Here in, we proposed to incorporate the dimerization concept in our design of new small molecules based on the traditional antibacterial agents—sulfonamide and trimethoprim. We anticipated that the dimeric ligand would be able to inhibit growth of S. aureus and MRSA with higher efficacy than the single antibacterial agent counterparts and potentially to overcome the resistance. Typically, heterodimeric ligand could take

advantage of its ability to chelate two different molecular targets or to sterically block the access of natural substrates while the single agents could not. Furthermore, the recent study with a phthalazine analogue of trimethoprim called RAB1 also supported our hypothesis. RAB1 was highlighted as the DHFR inhibitor, exhibiting broad-spectrum activity with exceptional effectiveness against *S. aureus* and MRSA (5). The reported X-ray crystal structure of *S. aureus* DHFR (saDHFR) with trimethoprim and with RAB1 suggested a unique shallow surface cavity that could be exploited in drug development for *S. aureus* infection. The co-crystal complex structure with DHFR also indicated that the F98Y mutation, which commonly found in TMP-resistance strains, would have minimal effects on saDHFR binding. Hence, saDHFR would perhaps be an excellent target for MRSA therapeutic development. We envisioned that dimeric display ligand would provide chances to access the unique shallow hydrophobic cavity of saDHFR to overcome TMP resistance.

We carried out this investigation by following these methodologies:

(i) Design and synthesis dimeric ligand displaying sulfonamide and trimethoprim.

We covalently linked trimethoprim to sulfonamide adducts via C-C bond formation. Heck coupling between the halogenated trimethoprim and terminal alkene on sulfonamide adduct would yield the desired dimer.

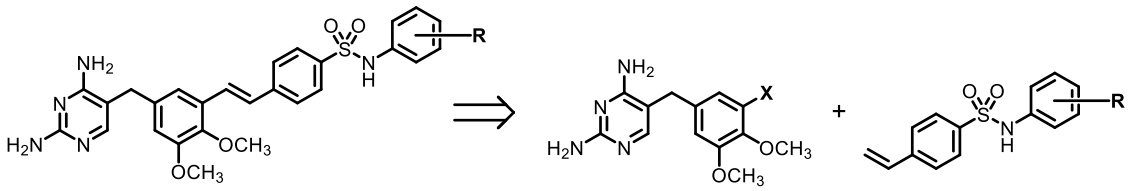


Figure 3. Retrosynthetic analysis of heterodimeric displayed sulfonamide and trimethoprim

The synthesis of terminal alkene-appended sulfonamide moiety could be synthesized from commercially available sodium vinylbenzenesulfonate. Transformation to acid chloride adduct, followed by reaction with amine derivatives of interest would afford terminal alkene-appended sulfonamide for further conjugation.

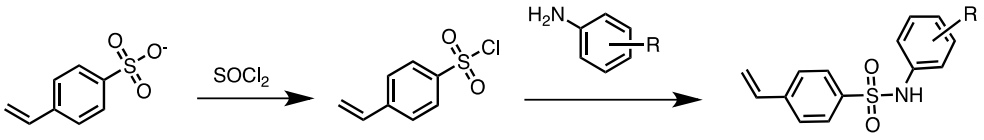


Figure 4. Synthetic plan of sulfonamide monomer

For the synthesis of trimethoprim derivative, commercially available vanillin would be undergo halogenation to afford halogenated vanillin, of which undergo methylation. The resulting aldehyde would then subjected to aldol condensation reaction with 3-morpholinopropanenitrile. The intermediate would be subsequently cyclized when treating with guanidinium salt under reflux, affording the desired halogenated trimethoprim for heterodimerization.

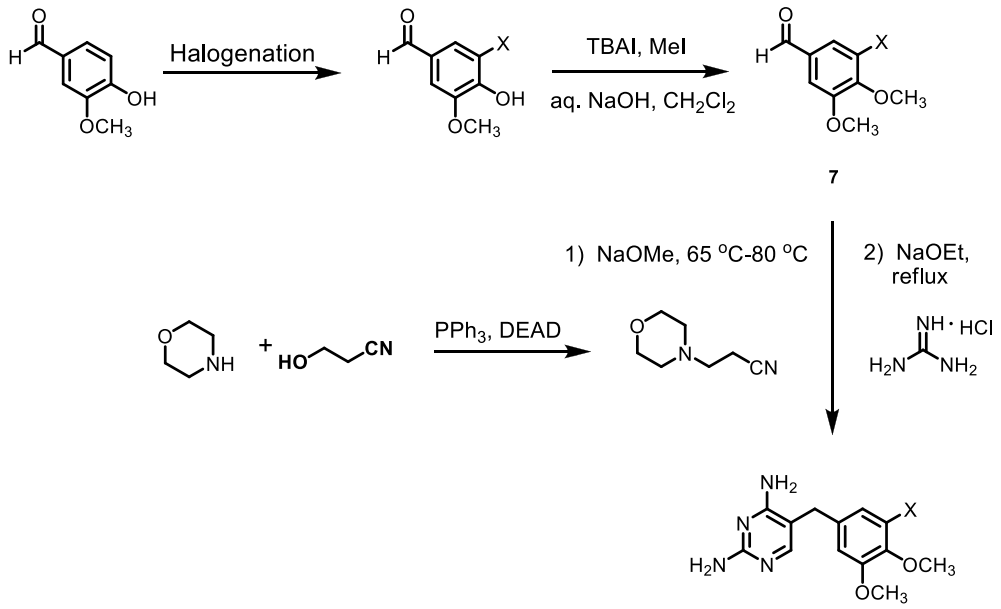


Figure 5. Synthetic plan of halogenated trimethoprim monomer

(ii) Determine the ability of synthetic dimer to inhibit growth of MSSA and global predominant multidrug-resistant *S. aureus*.

The synthetic dimer were subjected to susceptibility assays against methicillin susceptible *S. aureus* (MSSA) control strains, global predominant CA-MRSA PFGE strain USA300 (SF8300) and highly resistant HA-MRSA strain COL. Based on breakpoint of TMP and SMZ as suggested by CLSI, our preliminary results suggested that CA-MRSA strain SF8300 is resistant to SMZ while HA-MRSA COL strain is resistant to both antibacterial agents as well as to the combination therapy.

Two standard susceptibility assays—disk diffusion assay and microdilution assay—were performed to qualitatively and quantitatively suggest the ability of the dimeric compound to inhibit growths of pathogenic strains of study.

Heterodimeric ligand and its dissected components (TMP analogue and sulfonamide analogue) were first tested for the antimicrobial activity using disk diffusion method. The disk diffusion protocol were strictly followed the standard susceptibility protocol recommended by Clinical and Laboratory Standards Institute (CLSI). In brief, the sterilized Muller-Hinton Agar was prepared and poured into Petri dishes to acquire approximately 4 mm in thickness. After solidification, test strains was spread on the agar surface by using sterile swap. Spread inoculum was approximately 8 x10^7 CFU/mL. The inoculated plate was allowed to stand at room temperature for 5-10 min before the application of disks. Subsequently, small filter paper disks (6 mm) was placed on the agar (disks should be applied to the surface of agar plate within 15 min), and a solution of test compound was transferred onto each paper disk. After 18-20 h of incubation at 37ºC, the diameters of inhibition zones was measured in millimeters using sliding callipers. The experiments was performed at least three times to determine standard error of inhibition zone.

The microdilution susceptibility test was performed based on the previously reported protocol. The series concentration (2-fold dilution) of dimeric ligand and its dissected counterparts was prepared. A 180 µL

aliquot of each compound at different dilutions were transferred to sterilized 96 well-plates. To the aliquot of test compounds, 20 µL of test strain suspension with inoculum density of $2x10^5$ CFU/mL was added to each well. The assay plates were incubated at 37ºC for 20 h. After incubation, the optical density of each well was measured using microplate reader. The minimum inhibitory concentration (MIC) of each compound against tested strains and their minimum bactericidal concentration (MBC) will be determined for analysis.

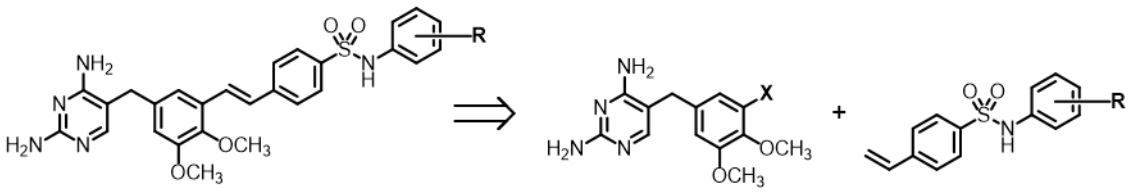
(iii) Investigate the mechanism of action underlying the growth inhibition activity.

The dimeric ligand will be tested for their ability to inhibit growth of MSSA and multidrug resistant S. aureus (SF8300 and COL strains) in the presence of PABA and thymidine supplemented in the growth medium to suggest whether this synthetic dimer exhibits growth inhibition activity via interfering with bacterial folate pathway.

Results:

(i) Design and synthesis dimeric ligand displaying sulfonamide and trimethoprim.

The heterodimeric ligand was be first simply designed to covalently linked trimethoprim and sulfonamide adducts via Heck coupling reaction, furnishing C-C bond linkage, as shown in the scheme below:

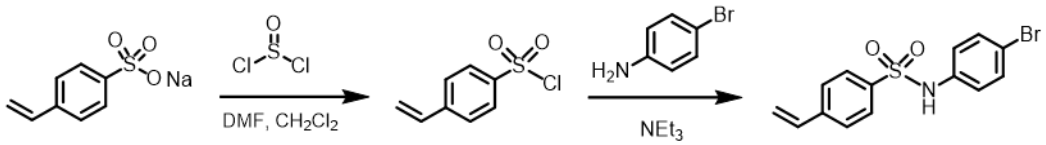


The rationale behind the design of the molecule would be described in the syntheses sections.

(a) Synthesis of Vinylbenzenesulfonamide

Our previous study suggested that electron-withdrawing substituent on benzene ring could enhance the ability of sulfonamide to inhibit bacterial growth (6). Therefore, the designed sulfonamide monomer should bear electron-withdrawing group at the position that not involve in the conjugation step for generating the dimer.

We started with the synthesis of vinylsulfonamides. Sodium vinylbenzenesulfonate was converted into vinylbenzenesulfonyl chloride by treating with thionyl chloride ($SOCl_2$), catalytic amount of DMF, in CH_2Cl_2. Vinylbenzenesulfonyl chloride readily reacted with aniline derivatives in CH_2Cl_2 resulting alkene-containing sulfonamide. Hence, we employed 4-bromoaniline to react with vinylbenzenesulfonyl chloride to yield the desired sulfonamide monomer.

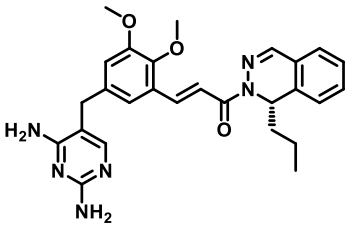


Scheme 1**:** Synthesis of sulfonamide monomer

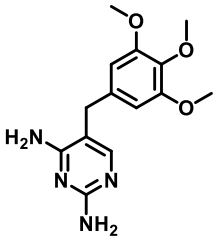
The synthesis was achieved, and the product was characterized by spectroscopic techniques.

(b) Synthesis of Halogenated Trimethoprim

The trimethoprim monomer for Heck coupling with sulfonamide synthesized requires halogen appending on the molecule. To design the site for halogenated, we rationally analyzed previously reported trimethoprim analogues. RAB1, a phthalazine analogue of trimethoprim, was reported as the DHFR inhibitor that exhibited broad spectrum activity with exceptional effectiveness against *S. aureus* and MRSA. (5) The reported X-ray crystal structure of *S. aureus* DHFR (saDHFR) with trimethoprim and with RAB1 suggested that RAB1 could bind at the same binding site as parented trimethoprim.

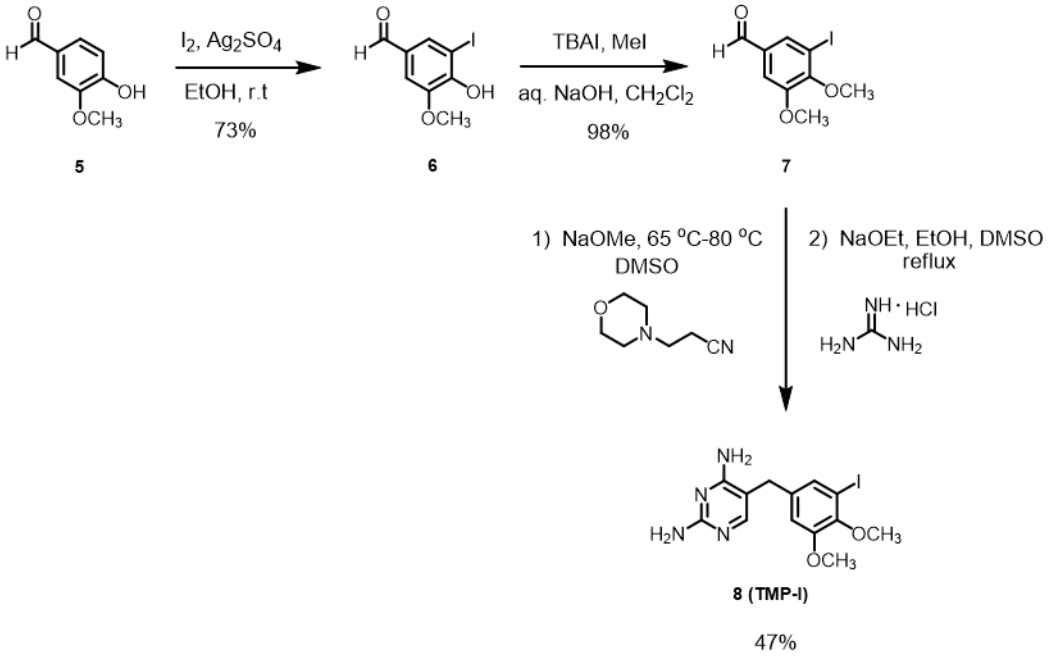


RAB1



Trimethoprim (TMP)

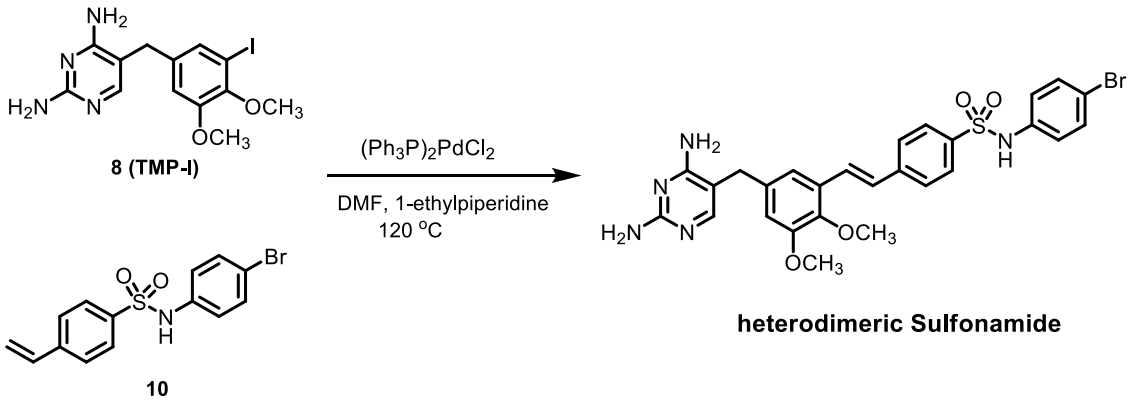
As suggested by the structure of RAB1, the methoxy substituent could be modified without perturbing the binding affinity to the DHFR protein. Therefore, we replaced the methoxy group with halogen for further coupling with the monomeric sulfonamide synthesized. The synthesis of halogenated trimethoprim could be achieved following this synthetic scheme shown below.



The syntheses of halogenated trimethoprim derivatives were based on previously reported method (7). In brief, an inexpensive commercially available vanillin was used as a starting material to undergo halogenation with various halogen sources. In this case, the iodination was accomplished by iodine in the presence of Ag_2SO_4. The halogenated vanillin was then subjected to methylation reaction by treating with methyl iodide, tetrabutylammonium iodide (TBAI) under basic condition. Condensation with 3-morpholinopropionitrile under basic condition yielded alkene product. Cyclization upon treating with guanidine afforded corresponding halogenated derivatives of trimethoprim. All purified synthetic products are characterized by ^{1}H-NMR, ^{13}C-NMR and resolution mass spectrometry.

(c) Synthesis of Heterodimeric Ligand

The vinylbenzenesulfonamide and iodo-trimethoprim was subjected to $(Ph_3P)_2PdCl_2$ catalyzed Heck coupling reaction to covalently link the two entities to yield heterodimer.

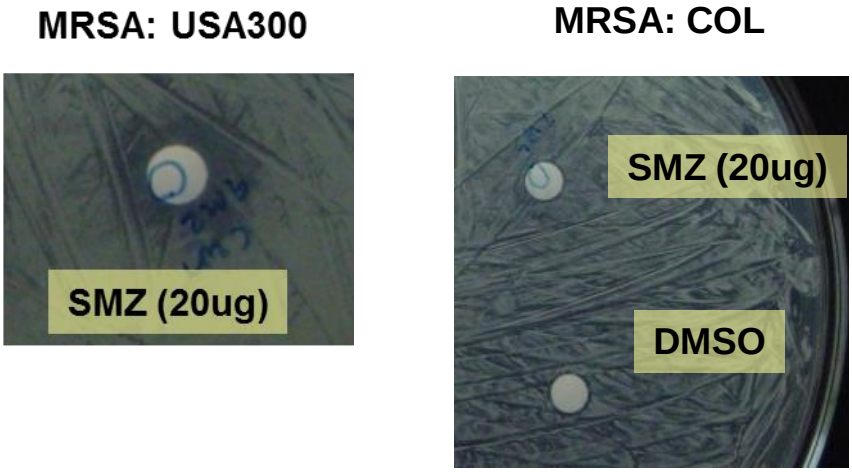


The final product was purified via column chromatography. The purified product was characterized by ^{1}H-NMR, ^{13}C-NMR and resolution mass spectrometry.

(ii) Evaluate susceptibility of MSSA and MRSA to monomeric vinylbenzene sulfonamides and halogenated trimethoprim

All compounds were first screen with disk diffusion assay. MSSA ATCC25923 was used as control strain. We chose community-acquired MRSA USA300 (strain SF8300) and hospital-acquired COL strain for our study. MRSA USA300 could infect healthy individuals in the community, easily spread, leading to transmission control problem. This particular strain is epidemically spread worldwide. Meanwhile, MRSA COL strain causes infection problem in healthcare institutes; the strain is well known to be highly resistant towards current antibiotics.

We tested susceptibility of these MSSA and MRSA strains to clinically used sulfonamide drug such as sulfamethoxazole (SMZ). Disk diffusion assay, as shown in the pictures below, suggested the SMZ could not inhibit growth of community-acquired MRSA (CA-MRSA) USA300 nor inhibit hospital-acquired MRSA (HA-MRSA) COL strain. No complete inhibition zone was observed.



The results of synthesized monomeric compounds were shown in the table below:

Compound	Complete Inhibition zone (mm)		
	ATCC 25923	MRSA: USA300	MRSA: COL
SMZ (sulfamethoxazole)	Incomplete (+)	Incomplete (+)	6
SXT (1:20 SMZ:TMP)	27.94	Incomplete (+++)	Incomplete (+++)
10 (25 µg)	7.86	6.13	6.73
TMP-I (5 µg)	19.55	19.87	Incomplete (+++)

The results indicated that clinically used combination therapy of trimethoprim and sulfamethoxazole (SXT) could inhibit growth of MSSA ATCC25923 effectively with complete inhibition zone >25 mm. The monomeric entities could also inhibit growth of MSSA; TMP-I showed better potency than vinylbenzenesulfonamide adduct (**10**). At the amount indicated, all compound synthesized along with known drugs could not inhibit MRSA COL strain while only TMP-I could inhibit MRSA: USA300. We further subjected these compounds to microdilution assay to determine minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). The results are shown below:

Compound	MIC /MBC		
	ATCC 25923	MRSA: USA300	MRSA: COL
Sulfamethoxazole	100	100	N/A
10	25/25	25/25	50/50
TMP-I	0.63/0.63	0.63/0.63	<5/<5
TMP	1.25/1.25	1.25/1.25	10/10

We found that MRSA:USA300 and COL strain are sulfamethoxazole resistant while COL strains is trimethoprim-resistant (suggested breakpoint <8 μg/mL). In correspond to the results from disk diffusion assay, TMP-I inhibit growth effectively (MIC of only 0.63 μM). Interestingly, iodo-TMP showed better ability to inhibit growth of MSSA and MRSA than TMP.

(ii) Evaluate susceptibility of MSSA and MRSA to heterodimeric displayed sulfonamides and trimethoprim

The heterodimeric ligand displaying trimethoprim and sulfonamide entities was tested for its ability to inhibit growth of *S. aureus*. Disk diffusion assay was first used to screen the compound. The results of the heterodimer along with the monomeric entities were shown in the table below.

Compound	Complete Inhibition zone (mm)		
	ATCC 25923	MRSA: USA300	MRSA: COL
SMZ (sulfamethoxazole)	Incomplete (+)	Incomplete (+)	6
SXT (1:20 SMZ:TMP)	27.94	Incomplete (+++)	Incomplete (+++)
sulfonamide monomer (25 μg)	7.86	6.13	6.73
TMP-I (5 μg)	19.55	19.87	Incomplete (+++)
TMP-I + sulfonamide monomer (25 μg)*	14.52	15.66	9.52
Heterodimeric Sulfonamide (5 μg)	11.15	13.62	9.96
Heterodimeric Sulfonamide (25 μg)	13.12	13.31	11.24

Based on the disk diffusion assay results, the heterodimeric compound exhibited its ability to inhibit growth of *S. aureus* in all strains as clear inhibition zone was observed when tested 25 μg. The combination of the two monomeric sulfonamide and trimethoprim at the same ratio of concentration as the clinically used combination therapy SXT was tested for comparison. The results showed that the combination of two monomeric entities synthesized could inhibit MRSA strains while the clinically used SXT could not inhibit both MRSA USA300 and COL strains. In addition, we also tested the heterodimeric compound at lower amount. We found that, even at 5 μg, the heterodimeric compound could inhibit all strains tested.

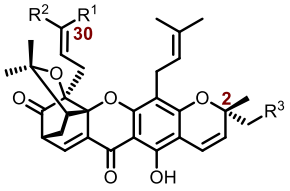
Compound	MIC /MBC		
	ATCC 25923	MRSA: USA300	MRSA: COL
Sulfamethoxazole	100	100	N/A
10	25/25	25/25	50/50
TMP-I	0.63/0.63	0.63/0.63	<5/<5
TMP	1.25/1.25	1.25/1.25	10/10
TMP-D1	5/5	5/5	N/A
Heterodimeric Sulfonamide	1.25/1.25	1.25/1.25	1.25/1.25

Subsequently, we determined MIC and MBC values to compare the efficacy of the heterodimeric compound in comparison to the monomeric entities. The results were shown in the table above. Based on the CLSI standards, the breakpoint of trimethoprim antibiotic is 8 µg/mL; therefore, the hospital acquired MRSA COL strain is trimethoprim-resistant. These results promisingly indicated that the heterodimeric displayed sulfonamide and trimethoprim could successfully inhibit growth of *S. aureus*, the resistant strain-MRSA PFGE strain type USA300 (SF8300), and also the multiple drugs-resistant MRSA COL strain with the excellent MIC of 1.25 µg/mL. In comparison with the monomeric TMP and sulfonamide, the heterodimeric compound exhibited significant enhancement in bacterial growth inhibitory activity (approximately 20 folds enhanced when compared to sulfonamide). In addition, the heterodimeric ligand also overcome the TMP resistance in COL strain. These results suggest the benefit of using multivalent display in the design of new antibacterial agents.

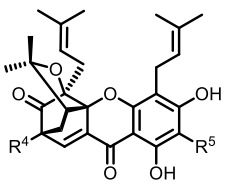
Additional Results (Change of Research Plan):

As stated in 18 months-progress report, we also survey the antibacterial activity of isolated natural products. This additional project was conducted in collaboration with the mentor, Prof. Vichai Reutrakul. We are particularly interested in caged xanthones, as they were isolated from Garcinia plant, which has been traditionally used to treat infected wounds. Along with this proposed project, we carried out the susceptibility testing of the caged xanthones derivatives. The experiments could be performed with similar protocols.

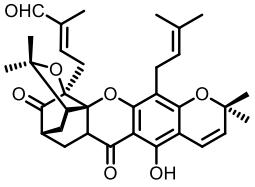
The prenylated caged xanthones **1'-9'** were isolated from resins and fruits of *Garcinia hanburyi hook. f.* The structure were shown below:



1: R^1 = -CH_3, R^2 = -CH_3, R^3 = -H
2: R^1 = -CH_3, R^2 = -CH_2OH, R^3 = -H
3: R^1 = -COOH, R^2 = -CH_3, R^3 = -H
4: R^1 = -COOH, R^2 = -CH_3, R^3 = Prenyl



5: R^4 = -H, R^5 = Prenyl
6: R^4 = -H, R^5 = Geranyl
7: R^4 = Prenyl, R^5 = -H
8: R^4 = -H, R^5 = H



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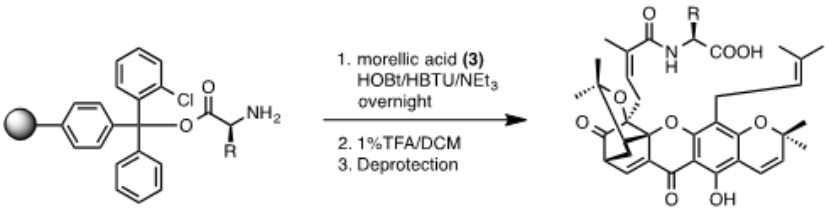
Natural caged xanthones **1'-9'** isolated from *G. hanburyi hook. f.* were first quantitatively screened for their ability to inhibit growth of MRSA USA300 strain SF8300 via disk diffusion assay. Methicillin susceptible *S. aureus* (MSSA) ATCC 25923 was used as control strain. Out of 9 caged xanthones, 5 compounds (**2'-5'**, **8'**) exhibited inhibition zones against both MSSA and MRSA strains while beta-lactam antibiotics such as ampicillin and oxacillin, as anticipated did not inhibit MRSA USA300. These 5 caged xanthones (**2'-5'**, **8'**) were further subjected to microdilution assay to quantitatively access their MIC and MBC. Among the natural caged xanthones assayed, morellic acid (**3'**) was the most potent MSSA and MRSA growth inhibitors with MIC of 12.5 μM, as shown in the Table below. Gambogic acid (**4'**) inhibited growth with comparable extent. The results indicated that prenyl moiety at C2 position was not significant for growth inhibition. The potency of isomorellinol (**2'**), 2-isoprenylforbesione (**5'**) and forbesione (**8'**) were dramatically decrease (MIC>200 μM) while the other caged adducts (**1'**, **6'-7'**, **9'**) tested could not inhibit growth of neither MRSA USA300 strain SF8300 nor MSSA control strain. Deoxymorellin (**1'**) and morellic acid (**3'**) share similarity in their structures with only minor exception at C-30. Interestingly, the anti-bacterial activities of these compounds were drastically different, highlighting the importance of carboxyl functionality at C-30 position.

Compounds	**Diameter of Clear zones (mm)**		**MIC (μM)**		**MBC (μM)**		**IC_{50} (μM)**
	ATCC 25923	**SF8300**	**ATCC 29213**	**SF8300**	**ATCC 29213**	**SF8300**	
Ampicillin (10 μg)	16.97±1.19[c]	6	1.56	>100	1.56	>100	-
Oxacillin (1 μg)	28.86±3.68[c]	6	0.62	>19.9	1.25	>19.9	-
Deoxymorellin **(1')**	6	6	-	-	-	-	1.42
Isomorellinol **(2')**	7.75±0.13	8.57±0.35	-	-	-	-	2.69
Morellic acid **(3')**	19.23±0.35	19.52±0.49	12.5	12.5	12.5	25	3.50
Gambogic acid **(4')**	16.59±0.51	17.29±0.77	12.5	25	25	50	3.40
2-Isoprenylforbesione **(5')**	7.56±0.12	7.20±0.53	400	>400	>400	>400	-
Deoxygamboginin **(6')**	6	6	-	-	-	-	-
Hanburin **(7')**	6	6	-	-	-	-	-
Forbesione **(8')**	7.86±0.12	7.97±0.11	200	>400	200	>400	-
Dihydroisomorellin **(9')**	6	6	-	-	-	-	-

Even though the natural morellic acid (**3'**) and gambogic acid (**4'**) possessed potent antibacterial activities, their high cytotoxicity hinder their potentials as bacterial infection therapeutics. The reported IC_{50} of morellic acid (**3'**) and gambogic acid (**4'**) are 3.5 μM and 3.4 μM, respectively. These IC_{50} values are above MIC and MBC values, suggesting that these compounds potentially would be toxic to treated cells. We, therefore, modified morellic acid (**3'**) in an effort to acquire the structure of caged xanthones that are potent antibacterial activities with low toxicity.

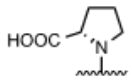
Based on our SAR analysis, the key structure for retaining anti-bacterial activity is carboxylic functionality at C-30. Replacing polar COOH substituent with less polar groups could drastically alter the antibacterial activity and could perhaps increase the cytotoxicity. Interestingly, N-(carboxymethyl) gambogiamide, GA3, was previously reported as potential candidate in cancer therapy (8). GA3 is a modification of –COOH functional group with natural amino acid glycine; this alteration in structure showed slightly reduce in cytotoxicity of gambogic acid yet enhanced solubility in aqueous solution. The declining trend in cytotoxicity with glycine conjugation at C-30 led us to further explore other amino acids conjugate at this particular position. We envisioned that amino acid conjugation analogues **3'b-m**, which still possess the carboxylic functionality, could perhaps retain potent antibacterial activity against MSSA and MRSA strains with less toxicity.

The synthetically modified morellic (**3'b-3'm**) were achieved via solid-phase synthesis and subjected to susceptibility testing with MRSA: USA300 and MSSA strains.



3b-m

3b: R = -$CH(CH_3)_2$
3c: R = -$CH_2C_6H_5$
3d: R = -CH_2CH_2COOBz
3e: R = -CH_2CH_2COOH
3f: R = -$CH_2CH_2SCH_3$
3g: R = -$CH_2CH(CH_3)_2$
3h: R = -CH_2OH
3i: R = -$CH(CH_3)CH_2CH_3$

3j: R = 

3k: R = -$CH_2CH_2CONHTrt$
3l: R = -$CH_2CH_2CONH_2$
3m: R = -$(CH_2)_4NH_2$

The results showed that compound **3'b**, **3'c**, **3'g**, **3'i**, and **3'j** could retain growth inhibitory activity in both strains. Interestingly, all of the morellic derivatives that inhibit bacterial growth were those that modified with amino acid that contain hydrophobic side chains. The inhibitory activity of compound **3'e**, which contains additional carboxylic acid functionality from glutamic acid side chain, was surprisingly diminished. The MIC of **3'b** and **3'c** are equivalent to that of natural morellic acid **3'**, indicating that these two derivatives could inhibit growth of MSSA and MRSA strains in comparable extent.

Compounds	Diameter of Clear zones (mm)		MIC (μM)		MBC (μM)		IC_{50} (μM)
	ATCC 25923	SF8300	ATCC 29213	SF8300	ATCC 29213	SF8300	
Morellic acid **(3')**	19.23±0.35	19.52±0.49	12.5	12.5	12.5	25	3.50
Methyl morellate **(3'a)**	6	6	-	-	-	-	-
3'b	19.99±0.43	22.24±2.06	12.5	25	12.5	25	29.8
3'c	17.07±2.03	16.27±2.33	12.5	25	12.5	25	12.2
3'd	6.53±0.92	6.27±0.46	-	-	-	-	-
3'e	6	6	-	-	-	-	-
3'f	7.09±1.13	9.53±1.13	-	-	-	-	-
3'g	16.52±2.49	18.34±2.43	25	25	25	50	15.5
3'h	6.09±0.18	6.28±0.56	-	-	-	-	-
3'i	15.91±0.79	19.35±1.60	25	25	25	50	24.5
3'j	13.08±0.37	15.91±1.60	50	100	100	>100	21.1
3'k	6.89±1.19	8.21±1.60	-	-	-	-	-
3'l	7.50±0.45	9.09±0.45	-	-	-	-	-

The cytotoxicity of derivatives that exhibited growth inhibitory activity (**3'b**, **3'c**, **3'g**, **3'i**, and **3'j**) was determined. When treated with human epithelial A549 cell line, the toxicity of these compounds was 10-fold lower than natural morellic acid. For instance, at MIC concentration, the derivative **3'b** could inhibit growth of bacteria and did not alter morphology of A549 cells. Meanwhile, the natural product morellic acid **3'** evidently damaged A549 cells at its MIC concentration. We also performed the cytotoxicity experiments with non-transformed cell line HEK293. Compound **3'b** also exhibited approximately 10-fold decrease in toxicity when compared with natural analogues, including morellic acid **3'**. This raises the potential for developing morellic acid derivatives as therapeutics for MRSA infection.

Conclusion and Discussion

In this study we determined the susceptibility of global epidemic MRSA strains to two classes of compounds—heterodimeric displayed sulfonamide trimethoprim and derivatives of natural isolated caged xanthones. We chose community-acquired MRSA USA300 (strain SF8300) and hospital-acquired COL strain for our study. MRSA USA300 is epidemically spread worldwide, causing healthcare problem. In addition, MRSA USA300, as the community-acquired strain, could infect healthy individuals in the community, and easily spread, leading to transmission control problem. Meanwhile, MRSA COL strain causes infection problem in healthcare institutes; the strain is well known to be highly resistant towards current antibiotics.

In the proposed project, we exploited the dimerization concept in our design of new small molecules based on the traditional antibacterial agents—sulfonamide and trimethoprim. The heterodimeric ligand was designed to covalently linked trimethoprim and sulfonamide adducts via Heck coupling reaction, furnishing C-C bond linkage. The heterodimeric compound could inhibit growth of *S. aureus* in all strains as clear inhibition zone was observed when tested 25 μg of heterodimer. We also tested the combination of the two monomeric sulfonamide and trimethoprim at the same ratio of concentration as the clinically used combination therapy SXT. The results showed that the combination of two monomeric entities synthesized could inhibit MRSA strains while the clinically used SXT could not inhibit both MRSA USA300 and COL strains. In addition, we also tested the heterodimeric compound at lower amount. We found that, even at 5 μg, the heterodimeric compound could inhibit all strains tested. MIC and MBC values of these compounds were determined. The results promisingly indicated that the heterodimeric displayed sulfonamide and trimethoprim could successfully inhibit growth of *S. aureus*, the resistant strain-MRSA PFGE strain type USA300 (SF8300), and also the multiple drugs-resistant MRSA COL strain with the excellent MIC of 1.25 μg/mL. In comparison with the monomeric TMP and sulfonamide, the heterodimeric compound exhibited significant enhancement in bacterial growth inhibitory activity (approximately 20 folds enhanced when compared to sulfonamide). In addition, the heterodimeric ligand also overcome the TMP resistance in COL strain. These results suggest the benefit of using multivalent display in the design of new antibacterial agents.

In addition,series of caged xanthones isolated from resins and fruit of G. hanburyi hook. f. Morellic acid (**3**') and gambogic acid (**4**') showed high potency in growth inhibitory activity with MIC of 12.5 μM. However, due to the high toxicity of these compounds, host cells could potentially be damaged when treated at MIC concentration, reducing their potentials as anti-bacterial agents for MRSA infection. We further modified the caged xanthone synthetically. Morellic acid derivative **3'b** was discovered to retain high potency in global epidemic MRSA: USA300 growth inhibitory activity. Synthetic modification in **3'b** led to significant reduction in toxicity that host cells could no longer be impaired when treated with **3'b** at its MIC.

References

1. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992 through June 2004. Am J Infect Control 2005; 32:470-480.
2. Mekviwattanawong S, Srifuengfung S, Chokepaibulkit K, Lohsiriwat D, Thamlikitkul V. Med Assoc Thai 2006; 89 (Suppl 5): S106-17.
3. Yoshida O, Nakamura J, Yamashiro H, Miura K, Hayashi S, Umetsu K, et al. New insight into the mode of action of vancomycin dimers in bacterial cell wall synthesis. MedChemComm. 2011;2(4):278-82.
4. Daniel DL, James BA, Burton GC, J Kevin J, Sharath SH, Koné K, Kevin MK, Martin SL, Edmund JM, John LP A Multivalent Approach to Drug Discovery for Novel Antibiotics The Journal of Antibiotics 2008; 61: 595–602.
5. Bourne CR, Barrow EW, Bunce RA, Bourne PC, Berlin KD, Barrow WW. Antimicrob Agents Chemother. 2010;54(9):3825-33.
6. Wanida Phetsang, Soraya Chaturongakul, Chutima Jiarpinitnun. Electron-withdrawing substituted benzenesulfonamides against the predominant community-associated methicillin-resistant Staphylococcus aureus strain USA300. Monatsh. Chem. 2013; 144:461–471.
7. Sirichaiwat C, Intaraudom C, Kamchonwongpaisan S, Vanichtanankul J, Thebtaranonth Y, Yuthavong Y. J Med Chem. 2004;47: 345-354.
8. Xie H, Qin YX, Zhou YL, Tong LJ, Lin LP, Geng MY, Duan WH, Ding J. Acta Pharmacologica Sinica 2009, 30, 346.

Output

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Caged xanthones: Potent inhibitors of global predominant MRSA USA300

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ABSTRACT

Total of 22 caged xanthones were subjected to susceptibility testing of global epidemic MRSA USA300. Natural morellic acid showed the strongest potency (MIC of 12.5 μM). However, its potent toxicity diminishes MRSA therapeutic potential. We synthetically modified natural morellic acid to yield 13 derivatives (**3a**–**3m**). Synthetically modified **3b** retained strong potency in MRSA growth inhibition, yet the toxicity was 20-fold less than natural morellic acid, permitting the possibility of using caged xanthones for MRSA therapeutic.

Garcinia hanburyi hook. f. belongs to Guttiferae family, commonly found in Southeast Asia and China.[1] In traditional medicine, the resin is applied as a laxative as well as therapeutic of infected wounds.[2] The prenylated caged xanthones **1**–**9** were isolated from resins and fruits of *Garcinia hanburyi hook. f.* (Fig. 1). The structures were elucidated based on spectroscopic analyses.[3] Besides their unique chemical structure, caged xanthones have received a high level of attention due to their potent cytotoxicity against various cultured mammalian cancer cell lines as well as drug-resistant cell lines at low micromolar concentration.[2–7] The mechanisms underlying the anticancer and antitumor activities were extensively studied.[8–12] Multiple targets and mechanisms were suggested to be accounting for the potent cytotoxicity of caged xanthones. These includes the induction of apoptosis via regulation of key proteins such as caspase3, Bax, and Bcl-2, suppression of telomerase activity, inhibition of cell metastasis by down-regulating the expression of metalloproteinases and integrin, and affecting angiogenesis.[13–17] In addition to potent cytotoxicity in cancer cells, naturally isolated caged xanthones from *G. hanburyi* also showed potent anti-HIV activities in reverse transcriptase assay with IC_{50} less than 50 μg/mL.[3] Antimicrobial activity of several caged xanthones were previously reported.[5,10,18–21] Caged xanthones were highlighted their potent growth inhibition in experimental staphylococcal infection in mice.[21] However, treatment of caged xanthones at high concentration resulted in side effects including the decline in cell growth and reduced blood cell count, suggesting that the potent cytotoxicity of these compounds hinders the potential of caged xanthones as anti-bacterial agent.

We are interested in developing antibacterial agents for global epidemic methicillin-resistant *Staphylococcus aureus* (MRSA), particularly MRSA PFGE strain type USA300 (SF8300). USA300 is known as community-acquired MRSA; its rapid transmission and resistance towards antibiotics lead to global healthcare problem.[22,23] Natural caged xanthones **1**–**9** isolated from *G. hanburyi hook. f.* were first quantitatively screened for their ability to inhibit growth of MRSA USA300 strain SF8300 via disk diffusion assay. Methicillin susceptible *S. aureus* (MSSA) ATCC 25923 was used as control strain. Out of 9 caged xanthones, 5 compounds (**2**–**5**, **8**) exhibited inhibition zones against both MSSA and MRSA strains while beta-lactam antibiotics such as ampicillin and oxacillin, as anticipated did not inhibit MRSA USA300. These 5 caged xanthones (**2**–**5**, **8**) were further subjected to microdilution assay to quantitatively access their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Among the natural caged xanthones assayed, morellic acid (**3**) was the most potent MSSA and MRSA growth inhibitors with MIC of 12.5 μM (Table 1).

Gambogic acid (**4**) inhibited growth with comparable extent. The results indicated that prenyl moiety at C-2 position was not significant for growth inhibition. The potency of isomorellinol (**2**), 2-isoprenylforbesione (**5**) and forbesione (**8**) were dramatically decrease (MIC > 200 μM) while the other caged adducts (**1**, **6**–**7**, **9**) tested could not inhibit growth of neither MRSA USA300 strain SF8300 nor MSSA control strain. Deoxymorellin (**1**) and morellic acid (**3**) share similarity in their structures with only minor

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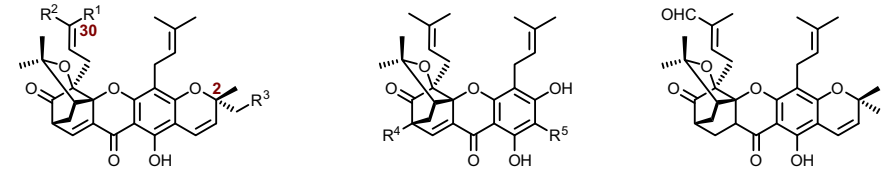

1: R^1 = -CH_3, R^2 = -CH_3, R^3 = -H
2: R^1 = -CH_3, R^2 = -CH_2OH, R^3 = -H
3: R^1 = -COOH, R^2 = -CH_3, R^3 = -H
4: R^1 = -COOH, R^2 = -CH_3, R^3 = Prenyl

5: R^4 = -H, R^5 = Prenyl
6: R^4 = -H, R^5 = Geranyl
7: R^4 = Prenyl, R^5 = -H
8: R^4 = -H, R^5 = H

9

Figure 1. Natural isolated caged xanthones (**1**–**9**).

Table 1
Antibacterial activities of caged-xanthones and their synthetic derivatives determined by disk diffusion[a] and microdilution susceptibility test[b]

Compounds	Diameter of clear zones (mm)		MIC (μM)		MBC (μM)		IC_{50} (μM)
	ATCC25923	SF8300	ATCC29213	SF8300	ATCC 29213	SF8300	
Ampicillin (10 μg)	16.97 ± 1.19[c]	6	1.56	>100	1.56	>100	–
Oxacillin (1 μg)	28.86 ± 3.68[c]	6	0.62	>19.9	1.25	>19.9	–
Deoxymorellin (**1**)	6	6	–	–	–	–	1.42
Isomorellinol (**2**)	7.75 ± 0.13	8.57 ± 0.35	–	–	–	–	2.69
Morellic acid (**3**)	19.23 ± 0.35	19.52 ± 0.49	12.5	12.5	12.5	25	3.50
Gambogic acid (**4**)	16.59 ± 0.51	17.29 ± 0.77	12.5	25	25	50	3.40
2-Isoprenylforbesione (**5**)	7.56 ± 0.12	7.20 ± 0.53	400	>400	>400	>400	–
Deoxygamboginin (**6**)	6	6	–	–	–	–	–
Hanburin (**7**)	6	6	–	–	–	–	–
Forbesione (**8**)	7.86 ± 0.12	7.97 ± 0.11	200	>400	200	>400	–
Dihydroisomorellin (**9**)	6	6	–	–	–	–	–
Methyl morellate (**3a**)	6	6	–	–	–	–	–
3b	19.99 ± 0.43	22.24 ± 2.06	12.5	25	12.5	25	29.8
3c	17.07 ± 2.03	16.27 ± 2.33	12.5	25	12.5	25	12.2
3d	6.53 ± 0.92	6.27 ± 0.46	–	–	–	–	–
3e	6	6	–	–	–	–	–
3f	7.09 ± 1.13	9.53 ± 1.13	–	–	–	–	–
3g	16.52 ± 2.49	18.34 ± 2.43	25	25	25	50	15.5
3h	6.09 ± 0.18	6.28 ± 0.56	–	–	–	–	–
3i	15.91 ± 0.79	19.35 ± 1.60	25	25	25	50	24.5
3j	13.08 ± 0.37	15.91 ± 1.60	50	100	100	>100	21.1
3k	6.89 ± 1.19	8.21 ± 1.60	–	–	–	–	–
3l	7.50 ± 0.45	9.09 ± 0.45	–	–	–	–	–

[a] 30 μg of test compounds in DMSO were used for disk diffusion testing. At least three independent experiments were performed and the average diameters of complete inhibition zones were reported with standard errors. Whatman disk has a diameter of 6 mm, therefore, average inhibition zone of 6 mm indicates inability to inhibit growth.
[b] Each compound was 2-fold diluted based on its stock concentration.
[c] The number indicates the diameter of visually complete inhibition zone. However, the compound also exhibited incomplete zone of inhibition.

exception at C-30. Interestingly, the anti-bacterial activities of these compounds were drastically different, highlighting the importance of carboxyl functionality at C-30 position. To verify the significance of carboxylic acid moiety, morellic acid **3** was methylated to the methyl ester (**3a**). The modified ester **3a** resulted in no inhibition activity. Hence, the carboxylic functionality is crucial for anti-bacterial activity. Even though the natural morellic acid (**3**) and gambogic acid (**4**) possessed potent antibacterial activities, their high cytotoxicity hinder their potentials as bacterial infection therapeutics. The reported IC_{50} of morellic acid (**3**) and gambogic acid (**4**) are 3.5 μM and 3.4 μM, respectively. These IC_{50} values are above MIC and MBC values, suggesting that these compounds potentially would be toxic to treated cells (see Supporting information). We, therefore, modified morellic acid (**3**) in an effort to acquire the derivative of caged xanthones that are potent antibacterial activities with low toxicity. Based on our SAR analysis, the key structure for retaining anti-bacterial activity is carboxylic functionality at C-30. To determine whether carboxylic functionality at C-30 is required for toxicity of the caged xanthones, deoxymorellin **1** and isomorellinol **2** were evaluated in comparison to morellic acid **3** and gambogic acid **4** as natural analogues **1** and **2** lack of –COOH substituent while **3** and **4** do contain –COOH at C-30. All compounds—deoxymorellin **1**, isomorellinol **2**, morellic acid **3** and gambogic acid **4** exhibited high cytotoxicity; however, deoxymorellin **1**, isomorellinol **2** were slightly more toxic than morellic acid **3** and gambogic acid **4**. These results along the susceptibility experiments suggested that replacing polar COOH substituent with less polar groups could drastically alter the antibacterial activity and could perhaps increase the cytotoxicity. Interestingly, N-(carboxymethyl) gambogiamide, GA3, was previously reported as potential candidate in cancer therapy.[24] GA3 is a modification of –COOH functional group with natural amino acid glycine; this alteration in structure showed slightly reduce in cytotoxicity of gambogic acid yet enhanced solubility in aqueous solution. The declining trend in cytotoxicity with glycine conjugation at C-30 led us to further explore other amino acids conjugate at this particular position. We envisioned that amino acid conjugation analogues **3b**–**m**, which still possess the carboxylic functionality, could perhaps retain potent antibacterial activity against MSSA and MRSA strains with less toxicity (Fig. 2).

The synthetically modified morellic (**3b**–**3m**) were achieved via solid-phase synthesis and subjected to susceptibility testing with MRSA: USA300 and MSSA strains. The results showed that compound **3b**, **3c**, **3g**, **3i**, and **3j** could retain growth inhibitory activity in both strains (Table 1). Interestingly, all of the morellic derivatives that inhibit bacterial growth were those that modified

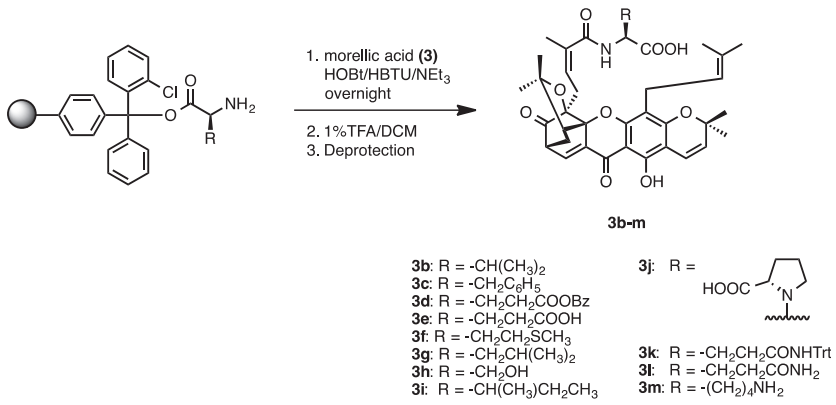

Figure 2. Syntheses of morellic acid derivatives (**3b**–**3m**).

with amino acid that contain hydrophobic side chains. The inhibitory activity of compound **3e**, which contains additional carboxylic acid functionality from glutamic acid side chain, was surprisingly diminished. The MIC of **3b** and **3c** are equivalent to that of natural morellic acid **3**, indicating that these two derivatives could inhibit growth of MSSA and MRSA strains in comparable extent. The cytotoxicity of derivatives that exhibited growth inhibitory activity (**3b**, **3c**, **3g**, **3i**, and **3j**) was determined. When treated with human epithelial A549 cell line, the toxicity of these compounds was 10-fold lower than natural morellic acid. For instance, at MIC concentration, the derivative **3b** could inhibit growth of bacteria and did not alter morphology of A549 cells. Meanwhile, the natural product morellic acid **3** evidently damaged A549 cells at its MIC concentration (see Supporting information). We also performed the cytotoxicity experiments with non-transformed cell line HEK293. Compound **3b** also exhibited approximately 10-fold decrease in toxicity when compared with natural analogues, including morellic acid **3** (see Supporting information). This raises the potential for developing morellic acid derivatives as therapeutics for MRSA infection.

The ability of bacterial cells to adhere and to invade into host cells is important for infection. Even though *S. aureus* is classically considered as extracellular bacteria, many evidence suggested that they could also persist inside host cells.[25] Interestingly, once they are uptaken inside host cells, not many current antibiotics could kill the bacteria.[26] Therefore, inhibition at the adhesion and invasion steps is essential. Several natural products, for instance *Curcuma longa L.*, could effectively prevent bacterial invasion to host cells.[27] Therefore, we further explored whether morellic derivative **3b** that exhibited potent anti-bacterial activity (MIC = 12.5 μM) against MSSA and MRSA could affect the ability of bacterial to invade host cells. *S. aureus* could invade into host cells within 15–90 min.[28] Human epithelial A549 cells were infected with *S. aureus* in the presence of testing compound **3b** at various concentrations for 2 h. We also attempted to compare the intracellular invasion inhibitory activity between the synthetic derivative **3b** with the natural caged xanthone morellic acid **3**. However, due to the high toxicity of morellic acid **3**, the concentration tested was necessitated to reduce to the concentration that does not damage A549 cells within 2 h of incubation. At 1.5 μM, morellic **3** could not inhibit invasion of *S. aureus* into host cells. Synthetic modified morellic acid derivative **3b** was found to inhibit invasion ability of *S. aureus* into host cells in concentration-dependent manner without perturbing morphology of host cells (see Supporting information). To determine whether the invasion results were not due to the effect of the compound on bacterial growth, the non-invading bacteria as well as invading bacteria were cultured. The results

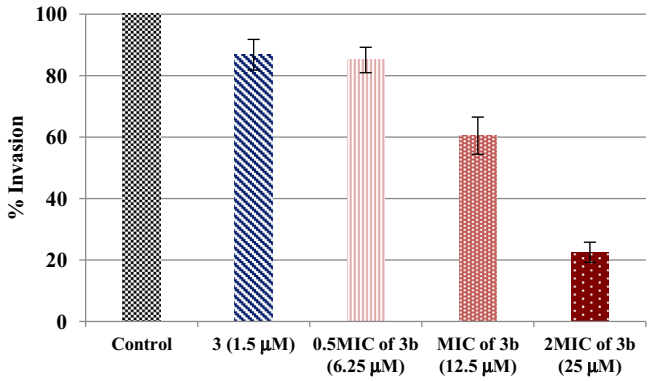

Figure 3. Ability of caged xanthones (**3b**) to inhibit bacterial invasion.

indicated that, when bacterial were exposed to caged xanthone analogue **3b** at MIC or even higher concentration for 2 h, compound **3b** did not perturb bacterial growth (see Supporting information). The mechanism underlying the inhibitory activity of caged xanthone **3b** in the invasion pathway is under investigation (Fig. 3).

In conclusion, in this study we determined the susceptibility of global epidemic MRSA USA300 strain SF8300 to series of caged xanthones isolated from resins and fruit of *G. hanburyi hook. f.* Morellic acid (**3**) and gambogic acid (**4**) showed high potency in growth inhibitory activity with MIC of 12.5 μM. However, due to the high toxicity of these compounds, host cells could potentially be damaged when treated at MIC concentration, reducing their potentials as anti-bacterial agents for MRSA infection. We further modified the caged xanthone synthetically. Morellic acid derivative **3b** was discovered to retain high potency in global epidemic MRSA: USA300 growth inhibitory activity. Synthetic modification in **3b** led to significant reduction in toxicity that host cells could no longer be impaired when treated with **3b** at its MIC. We discovered that caged xanthone **3b** could disrupt intracellular invasion of *S. aureus*. How caged xanthone **3b** could perturb intracellular invasion of bacteria should be further studied.

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Supplementary data

Supplementary data (synthetic methods and experimental details, including morphological changes of A549 and HEK293 in the presence of caged xanthones) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.05.030.

References and notes

1. Anantachoke, N.; Tuchinda, P.; Kuhakarn, C.; Pohmakotr, M.; Reutrakul, V. *Pharm. Biol.* **2012**, *50*, 78.
2. Panthong, A.; Norkaew, P.; Kanjanapothi, D.; Taesotikul, T.; Anantachoke, N.; Reutrakul, V. *J. Ethnopharmacol.* **2007**, *111*, 335.
3. Reutrakul, V.; Anantachoke, N.; Pohmakotr, M.; Jaipetch, T.; Sophasan, S.; Yoosook, C.; Kasisit, J.; Napaswat, C.; Santisuk, T.; Tuchinda, P. *Planta Med.* **2007**, *73*, 33.
4. Asano, J.; Chiba, K.; Tada, M.; Yoshii, T. *Phytochemistry* **1996**, *41*, 815.
5. Rukachaisirikul, V.; Phainuphong, P.; Sukpondma, Y.; Phongpaichit, S.; Taylor, W. C. *Planta Med.* **2005**, *71*, 165.
6. Tao, S. J.; Guan, S. H.; Wang, W.; Lu, Z. Q.; Chen, G. T.; Sha, N.; Yue, Q. X.; Liu, X.; Guo, D. A. *J. Nat. Prod.* **2009**, *72*, 117.
7. Han, Q.; Yang, L.; Liu, Y.; Wang, Y.; Qiao, C.; Song, J.; Xu, L.; Yang, D.; Chen, S.; Xu, H. *Planta Med.* **2006**, *72*, 281.
8. Han, Q. B.; Wang, Y. L.; Yang, L.; Tso, T. F.; Qiao, C. F.; Song, J. Z.; Xu, L. J.; Chen, S. L.; Yang, D. J.; Xu, H. X. *Chem. Pharm. Bull.* **2006**, *54*, 265.
9. Wang, J.; Zhao, L.; Hu, Y.; Guo, Q.; Zhang, L.; Wang, X.; Li, N.; You, Q. *Eur. J. Med. Chem.* **2009**, *44*, 2611.
10. Ren, Y.; Lantvit, D. D.; De Blanco, E. J. C.; Kardono, L. B. S.; Riswan, S.; Chai, H.; Cottrell, C. E.; Farnsworth, N. R.; Swanson, S. M.; Ding, Y.; Li, X. C.; Marais, J. P. J.; Ferreira, D.; Kinghorn, A. D. *Tetrahedron* **2010**, *66*, 5311.
11. Wang, X.; Lu, N.; Yang, Q.; Dai, Q.; Tao, L.; Guo, X.; Guo, Q.; You, Q. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2438.
12. Chantarasriwong, O.; Batova, A.; Chavasiri, W.; Theodorakis, E. A. *Chem. Eur. J.* **2010**, *16*, 9944.
13. Zhang, H.-Z.; Kasibhatla, S.; Wang, Y.; Herich, J.; Guastella, J.; Tseng, B.; Drewe, J.; Cai, S. X. *Bioorg. Med. Chem.* **2004**, *12*, 309.
14. Qin, Y.; Meng, L.; Hu, C.; Duan, W.; Zuo, Z.; Lin, L.; Zhang, X.; Ding, J. *Mol. Cancer Ther.* **2007**, *6*, 2429.
15. Qi, Q.; Gu, H.; Yang, Y.; Lu, N.; Zhao, J.; Liu, W.; Ling, H.; You, Q. D.; Wang, X.; Guo, Q. *J. Mol. Med.* **2008**, *86*, 1367.
16. Zhao, J.; Qi, Q.; Yang, Y.; Gu, H.-Y.; Lu, N.; Liu, W.; Wang, W.; Qiang, L.; Zhang, L.-B.; You, Q.-D.; Guo, Q.-L. *Eur. J. Pharmacol.* **2008**, *589*, 127.
17. Yi, T.; Yi, Z.; Cho, S. G.; Luo, J.; Pandey, M. K.; Aggarwal, B. B.; Liu, M. *Cancer Res.* **2008**, *68*, 1843.
18. Rukachaisirikul, V.; Kaewnok, W.; Koysomboon, S.; Phongpaichit, S.; Taylor, W. C. *Tetrahedron* **2000**, *56*, 8539.
19. Sukpondma, Y.; Rukachaisirikul, V.; Phongpaichit, S. *Chem. Pharm. Bull.* **2005**, *53*, 850.
20. Nageswara Rao, K. V.; Narasimha Rao, P. L. *Experientia* **1961**, *17*, 213.
21. Rao, K. V.; Rao, P. L. *Indian J. Exp. Biol.* **1967**, *5*, 101.
22. Tenover, F. C.; Goering, R. V. *J. Antimicrob. Chemother.* **2009**, *64*, 441.
23. Otto, M. *Cell. Microbiol.* **2012**, *14*, 1513.
24. Xie, H.; Qin, Y. X.; Zhou, Y. L.; Tong, L. J.; Lin, L. P.; Geng, M. Y.; Duan, W. H.; Ding, J. *Acta Pharmacol. Sin.* **2009**, *30*, 346.
25. Lowy, F. D. *Trends Microbiol.* **2000**, *8*, 341.
26. Krut, O.; Sommer, H.; Krönke, M. *J. Antimicrob. Chemother.* **2004**, *53*, 167.
27. Kim, K. J.; Yu, H. H.; Cha, J. D.; Seo, S. J.; Choi, N. Y.; You, Y. O. *Phytother. Res.* **2005**, *19*, 599.
28. Edwards, A. M.; Massey, R. C. *J. Vis. Exp.* **2011**, *49*, 2693.